Published online August 1, 2005

Total Dietary Fiber Variability in a Cross Section of *Crotalaria juncea* Genetic Resources

J. B. Morris* and S. E. Kays

ABSTRACT

Sunn hemp seeds contain several phytochemicals including fiber with potential phytopharmaceutical use. A study of 15 sunn hemp, *Crotalaria juncea* L., accessions demonstrated that total dietary fiber and protein content are significantly variable. As dietary fiber increases in the sunn hemp seed, protein decreases. Differences in total dietary fiber suggest that sufficient variability exists in this trait to allow enhancement in sunn hemp cultivars through plant breeding.

Sunn hemp is a member of the Leguminosae (Fabaceae) family and economically is the most important species of the genus *Crotalaria,* which consists of about 550 species. Sunn hemp originated in India and is characterized as a short day erect herbaceous plant with vegetative parts covered by short downy trichomes (Purseglove, 1981). The stems are green, cylindrical, ribbed, and 1 to 5 m in height; the leaves are simple and spirally arranged with minute stipules; and the roots are long with a strong tap root and numerous lateral roots. The inflorescence is a terminal open raceme with minute linear bracts and showy flowers. Indeterminate flowering occurs with dark yellow corollas having red to purple streaks on the dorsal surface. The fruit is a soft, hairy, inflated pod containing 10 to 15 seeds that are small, flat, kidney shaped, and dark gray to black (Purseglove, 1981). Sunn hemp has a $2n$ chromosome number of 16 and is predominantly cross-pollinated. Self-pollination takes place only after the stigmatic surface has been stimulated by a bee or mechanically pollinated.

Because of the fibers produced in the stem, one of the primary uses of sunn hemp is for the manufacture of twine, cord, marine cordage, fishing nets, matting, sacking, and paper (Purseglove, 1981; Cook and White, 1996). For certain fiber properties, sunn hemp is superior to kenaf (*Hibiscus cannabinus* L.) as a fiber crop. It is also superior because of its resistance to root-knot nematodes [*Meloidogyne incognita* (Koford & White) Chitwood] and has the advantage of being a soil-improving crop via nitrogen fixation (Dempsey, 1975).

Fiber is found throughout the sunn hemp plant, including the seeds (Anonymous, 1921). Total fiber content of materials is measured as total dietary fiber (AOAC, 2000d), which by definition includes plant nonstarch polysaccharides, oligosaccharides, resistant starch, and lignin (AACC, 2001). Lignin is present in the water insoluble fraction of fiber and the polysaccharides can be in either the water soluble or insoluble fraction depending on their structure. In addition to its commercial uses, fiber is beneficial to human health; high fiber diets have been associated with reduced calorie intake, improved digestive function (Anderson et al., 1994), reduced risk of coronary heart disease (Bazzano et al., 2003), and protection against the progression of atherosclerosis (Wu et al., 2003). More specifically, the insoluble fraction of total dietary fiber is required for normal lower intestinal function (Anderson et al., 1994), while diets high in certain types of soluble fiber are associated with lowering of serum cholesterol and the glycemic index (Anderson and Hannah, 1999; Battilana et al., 2001; Brown et al., 1999; Chandalia et al., 2000). Whereas the presence of fiber in sunn hemp seeds has been reported (Anonymous, 1921), the genotypic variability of fiber and the type of fiber present in the seeds is unknown.

Although several phytochemicals in sunn hemp seeds have potential use as phytopharmaceuticals, alkaloids in the seeds appear to be toxic to certain animals (Nobre et al., 1994; Zhang, 1985). These may represent a risk factor for certain phytochemical extracts, and if whole seeds are consumed in large quantities.

The USDA, ARS, Plant Genetic Resources Conservation Unit (PGRCU) maintains 22 sunn hemp accessions from Angola, Brazil, Guadeloupe, India, Mozambique, Myanmar, Nigeria, Pakistan, South Africa, former Soviet Union, Sri Lanka, Taiwan, and the USA. Although the general composition of sunn hemp seeds has been reported (Anonymous, 1921), the genetic variability for fiber, protein, and dry matter is unknown. This study was conducted to determine the genotypic variability for total dietary fiber, protein, and dry matter among 15 geographically diverse sunn hemp genotypes. In addition, the ratio of soluble to insoluble dietary fiber and the fat and ash content of the seeds were determined.

MATERIALS AND METHODS

To test viability of the seeds and obtain morphological characteristics, 15 accessions of sunn hemp seeds from the USDA, ARS, PGRCU (Griffin, GA) were direct seeded to regeneration plots in a field of clayey, kaolinitic, thermic Typic Kanhapludult soil series at Griffin, GA, during the first week of June 2002. Each plot of each accession consisted of one 6 m row with 6 m between rows. Plots were irrigated as necessary. Data were recorded on leafing, branching characteristics, plant height, and width.

Crotalaria juncea seeds for composition analysis were also obtained from the USDA, ARS, PGRCU (Griffin, GA). Seeds in the collection had been regenerated in the same location under uniform and optimum conditions. Six accessions from the collection were analyzed for preliminary evaluation in 2000 using 100 seeds for each accession. In 2002, 15 accessions

J.B. Morris, USDA-ARS, PGRCU, 1109 Experiment St., Griffin, GA 30223-1797; S.E. Kays, USDA, ARS, QARU, P.O. Box 5677, Athens, GA 30604-5677. Received 27 Oct. 2004. *Corresponding author (bmorris@ars-grin.gov).

Published in Crop Sci. 45:1826–1829 (2005).
Plant Genetic Resources
doi:10.2135/cropsci2004.0630

677 S. Segoe Rd., Madison, WI 53711 USA

Table 1. Morphological description and origin for the *Crotalaria juncea* accessions evaluated.†

PI	Origin	Average seed weight	Branch‡	Foliage§	Plant height	Plant width
		mg^{-1}			cm	cm
189043	Nigeria	25.0	many	many	90	90
207657	Sri Lanka	40.0	many	many	174	80
234771	Nigeria	29.1	medium	many	200	76
248491	Brazil	40.6				
250485	India	46.0	medium	medium	140	100
250486	India	43.8	medium	medium	100	120
250487	India	44.3	medium	medium	70	170
295851	Brazil	48.6	medium	medium	165	90
314239	Fmr Soviet Union	38.3	many	many	180	130
322377	Brazil	35.0	few	sparse	180	100
337080	Brazil	36.0	medium	medium	257	70
346297	India	43.7	medium	medium	200	140
391567	S. Africa	37.3	medium	medium	200	75
426626	Pakistan	40.8	few	sparse	220	200
561720	Brazil	50.0				

† Calculated from 300–500 seeds.
‡ Many branches = ≥67%; medium = 34% to 66%; and few = ≤33% of each plant producing branches based on visual observation.
§ Many = ≥67%; medium = 34% to 66%; and sparse = ≤33% of each plant producing foliage based on visual observation.

were analyzed (including the six from 2000) using 300 to 500 seeds from each accession (see Table 1 for morphological descriptions of each *C. juncea* accession). In 2002, the seeds received for each accession were weighed and the average weight per seed calculated (Table 1). The seeds from each accession, within years, were ground in a Spex mixer/mill (Spex Industries Inc., Metuchen, NJ). The seeds were ground in batches of approximately 100 seeds and each batch milled three times for three minutes (with cooling of the mill each time) to pass a 1000-μm screen. Within years, batches from the same accession were combined, mixed thoroughly, and stored in low-density polyethylene bags at –28°C until analysis.

All analyses were performed in duplicate. Dry matter was determined immediately after grinding using AOAC Method 945.15 (AOAC, 2000b) in a forced air oven at 105°C. AOAC Method 991.43 (AOAC, 2000d), an enzymatic gravimetric method for total dietary fiber (TDF), was used to analyze fiber. This TDF method includes both soluble and insoluble fractions of fiber in the measurement, in contrast to the crude fiber, acid detergent fiber and neutral detergent fiber methods, which do not include all fiber fractions (Dreher, 1987). The enzymes used for the TDF assay were obtained from Megazyme International Ireland Inc. (Bray, Ireland). Protein was determined by combustion analysis according to AOAC Method 992.23 (AOAC, 2000e) with the LECO FP2000 combustion analysis system (Leco Corporation, St. Joseph, MI). The nitrogen-to-protein conversion factor used was 6.25. Crude fat was determined by AOAC Method 945.16 (AOAC, 2000c) with petroleum ether as the solvent. AOAC Method 923.03 (AOAC, 2000a) was used to determine ash with a muffle furnace set at 550°C. The soluble and insoluble fiber fractions of sunn hemp accessions were measured by AOAC Method 991.43 (AOAC, 2000d). Assays were repeated if the differences between duplicates exceeded 1.7% in absolute values for TDF (one sample only) and 0.3% in absolute values for dry matter (no samples), protein (two samples), fat (no samples), and ash (two samples).

One-way analysis of variance using SAS 2004 was used to analyze data and Fisher's protected LSD test was used to separate means within years. Correlation analysis was performed on parameters within each year

Table 2. Preliminary evaluation of seed dry matter, protein and total dietary fiber (TDF) content among 6 *Crotalaria juncea* genotypes in 2000.

PI	Percentage dry matter	Percentage† protein	Percentage† TDF dry
314239	89.4 c	47.0 ab	37.3 d
207657	89.7 b	45.0 b	38.4 d
250486	89.6 b	49.0 a	38.9 d
426626	89.5 bc	44.0 b	41.3 c
322377	90.0 a	38.5 c	44.4 b
189043	88.7 d	28.0 d	48.8 a

† Protein and TDF are expressed on a dry matter basis; means followed by the same letter within columns indicate no difference at $P = 0.01$ by Fisher's protected LSD test ($N = 2$).

RESULTS AND DISCUSSION

Morphological descriptions and the average seed weights of the *C. juncea* accessions are shown in Table 1. The accessions that produced the most biomass were PI 189043, PI 207657, and PI 314239. However, average seed weights were highest in PI 561720, PI 295851, and PI 250485 producing 50, 48.6, and 46 mg, respectively. The largest plants were produced in accessions PI 426626, and PI 346297 with dimensions of 220 cm tall × 200 cm wide, and 200 cm tall × 140 cm wide, respectively.

Dry matter was significantly variable among accessions in 2000; however, the range in dry matter was narrow (88.7–90.0%). In addition, significant variation for total dietary fiber and protein, on a dry weight basis, was observed in 2000 (Table 2). The sunn hemp accession PI 189043 produced significantly more TDF (48.8%) than all other accessions and had significantly lower protein (28.0%) and dry matter content (88.7%) than the other accessions. Total dietary fiber in both PI 322377 (44.4%) and PI 426626 (41.3%) was significantly higher than in the remaining three accessions (37.3–38.9% TDF). PI 250486 produced the highest amount of protein (49.0%) and was one of the accessions with the lowest amount of TDF (38.9%). A high negative correlation was observed between TDF and protein content (-0.92, $p < 0.01$). Thus, as TDF content of the seeds increased, protein content decreased. When the insoluble and soluble dietary fiber fractions of three accessions with low, medium, and high TDF content were compared the soluble fiber fraction was found to be a substantial portion (26.4 ± 1.8, 33.2 ± 1.2, and 41.4 ± 1.6%, mean ± standard deviation) of the total fiber (Table 3).

Significant variability was also observed for TDF and protein content in the 15 accessions analyzed in 2002 (Table 4). Similar results were observed compared to the analysis conducted in 2000. Once again, PI 189043 produced significantly more total dietary fiber (51.7%)

Table 3. Determination of soluble and insoluble fractions of total dietary fiber† of three *Crotalaria juncea* genotypes evaluated in 2000.

PI	Insoluble fraction (% of TDF)	Soluble fraction (% of TDF)
207657	73.6 ± 2.4	26.4 ± 1.8
426626	58.6 ± 1.6	41.4 ± 1.6
189043	66.8 ± 2.3	33.2 ± 1.2

† Calculated on a dry weight basis; means ± standard deviation, $N = 2$.

Table 4. Mean percentages for seed dry matter, ash, protein, fat, and total dietary fiber (TDF) content for 15 *Crotalaria juncea* genotypes evaluated in 2002.†

PI	Percentage dry matter	Percentage ash	Percentage protein	Percentage fat	Percentage TDF
314239	89.8 b	4.02 ef	44.2 a	3.58 f	38.8 h
250485	88.5 j	4.38 bc	41.1 h	3.91 bcde	39.2 gh
207657	89.7 bc	4.55 a	42.3 e	3.86 cde	39.2 gh
250487	89.4 def	4.33 bc	42.8 d	3.92 bcd	39.6 gh
295851	89.6 bcd	3.86 gh	43.1 c	4.01 bcd	40.2 gh
250486	88.9 h	4.08 ef	43.5 b	3.56 f	40.2 g
346297	89.5 de	4.26 cd	41.5 g	4.37 a	40.5 fg
337080	89.4 ef	4.29 cd	38.7 l	3.82 de	41.7 ef
248491	88.7 i	4.59 a	42.0 f	3.23 g	41.9 ef
426626	90.1 a	3.80 hi	40.6 i	3.89 bcde	42.7 de
561720	89.2 g	4.47 ab	40.3 j	4.02 bcd	42.8 de
391567	89.3 fg	4.16 de	39.5 k	3.72 ef	43.3 cd
322377	90.0 a	4.32 bc	37.5 m	4.04 bc	44.2 bc
234771	89.5 cde	3.98 fg	37.1 n	4.07 b	44.7 b
189043	88.1 k	3.64 i	26.2 o	2.88 h	51.7 a

† Ash, protein, fat, and TDF expressed on a dry matter basis; means followed by the same letter within columns indicate no difference at $P = 0.01$ by Fisher's protected LSD test ($N = 2$).

than all other accessions, had significantly lower protein (26.2%), dry matter (88.1%) and fat (2.88%) than all other accessions and had the lowest ash content (3.64%). The next in order of dietary fiber content was PI 234771 (44.7%), which contained significantly higher TDF than the 12 accessions with the lowest TDF. Accessions PI 322377 (44.2%), 391567 (43.3%), 561720 (42.8%), and 426626 (42.7%) followed and had significantly higher TDF than the 7 accessions that were the lowest in TDF (38.8-40.5%). The accessions PI 314239, 250486, 295851, and 250487 were significantly higher in protein than all other accessions. Again a high negative correlation was observed between TDF and protein content (−0.94) (Table 5) and consistent trends were observed in both years for TDF and protein for corresponding accessions. The seeds of accession PI 189043 are considerably smaller than the seeds of all other accessions (Table 1) and the TDF is higher (Tables 2 and 4) than other accessions. The overall correlation between seed weight and TDF content among the accessions was −0.73 ($p < 0.01$). This may be due to a higher proportion of seed coat in smaller seeds.

Biologically significant variation in TDF, soluble fiber, and protein content occurs for genetic manipulation of these components. Sunn hemp lines could possibly be selected for regeneration and extraction of dietary fiber for use as an ingredient to boost dietary fiber levels in processed food products. Sunn hemp has a considerable advantage over other crops in this respect because of the large amount of total fiber and soluble fiber present in the seeds. The proportion of soluble fiber in the accessions analyzed was high, 26, 33, and 41% of total

Table 5. Correlation coefficients for dry matter, protein, fat, and total dietary fiber (TDF) among 15 *Crotalaria juncea* genotypes in 2002.

	Protein	Fat	TDF
Dry matter	0.44**	0.62**	−0.36*
Protein		0.45**	−0.94**
Fat			−0.48**

* Indicates significance at the $P = 0.05$ level of probability.
** Indicates significance at the $P = 0.01$ level of probability.

fiber. In comparison, barley (*Hordeum vulgare* L.), a cereal grain considered to have a relatively high soluble fiber content, contains 6 to 18% total dietary fiber (dry weight basis) with 29 to 62% (depending on the cultivar) in the form of soluble fiber; rolled oats, also considered a good source of TDF and soluble fiber, have 11% TDF with approximately 50% occurring as soluble fiber (Kays, unpublished data). Thus, compared with cereals, *C. juncea* can be a rich source of total and soluble dietary fiber; however, the type of soluble fiber present has not yet been determined. Identification of the types of soluble fiber found in sunn hemp could be the focus of future research.

There have been several reports on toxicity of the seeds of sunn hemp. According to some reports, they have been fed to cattle and sheep without ill effects becoming evident (Anonymous, 1921; Timon, 1929), although they are known to contain alkaloids. Another report states that the seeds are toxic to humans if large quantities are eaten (Russell et al., 1997). Toxicity, resulting in hepatic lesions and death, has been reported in horses and pigs when sunn hemp seeds comprised a substantial portion of their diet (Nobre et al., 1994; Zhang, 1985). Pyrrolizidine alkaloids have been identified as the critical toxic component with toxicity in pigs principally attributed to the alkaloid trichodesmine (Zhang, 1985). The variation in toxic alkaloids among accessions of sunn hemp is not known and should be researched if components of the seeds are to be consumed. It may be possible to use the seeds of sunn hemp as a source of dietary fiber if the fiber extraction process can be accomplished without coextraction of any toxic components.

Although statistically significant differences occur among accessions for dry matter, fat, and ash contents, biological significance is not evident as the ranges in these components are small. For example, in 2002 the ranges in dry matter, fat and ash were 88.1 to 90.1%, 2.88 to 4.37%, and 3.64 to 4.59%, respectively (Table 4).

The composition of the remaining dry matter of sunn hemp seeds is assumed to be predominantly starch with micro quantities of a variety of phytochemicals such as pigments, vitamins, phytosterols, isoflavones, and alkaloids. The approximate range in the remaining material for the 15 accessions analyzed in 2002 was calculated by difference from the data in Table 4 (Merrill and Watt, 1973) and is 8.3 to 15.6%. Accession PI 189043 contains significantly more material in the remaining portion than all other accessions (15.6%) and if this accession is omitted the variation becomes quite small, i.e., 8.3 to 11.5%. If the majority of the remaining material is assumed to be starch, it appears that accession PI 189043 has the genetic propensity for much greater production of carbohydrate (starch and fiber) than the other accessions.

Results for sunn hemp composition agree with a previous report on composition for moisture, crude protein, fat, and ash. A 1921 report (Anonymous, 1921) gives the composition as moisture 8.6%, crude protein 34.6%, fat 4.3%, and ash 3.3%. For fiber the percentage was much lower than the current study and was reported as

8.8%. The method of measurement of fiber was not given by the authors but was likely to have been the method for crude fiber because that was the only method available at that time. This method measures a portion of the cellulose and a small amount of both the lignin and hemicelluloses present (Dreher, 1987). The remainder of the cellulose, lignin, soluble, and insoluble hemicelluloses and any other soluble fibers present would have been in the unmeasured portion of the seed, which was calculated by difference as 41.1% and assumed by the authors to be starch. The current method for total dietary fiber was developed in 1991 (AOAC, 2000d) and was not in use at that time.

CONCLUSIONS

Significant variability among 15 *C. juncea* accessions has been observed. This variability shows which sunn hemp genotypes produce the greatest amount of TDF and protein and could assist breeders and other scientists in developing superior cultivars with higher amounts of TDF or protein.

This study identified a large amount of genetic variation for dietary fiber, a large portion of which is soluble fiber, in sunn hemp seeds. This information can provide direction to accessions of sunn hemp germplasm as source plants for extraction and enhancement of fiber as a nutraceutical. However, toxicity in sunn hemp seeds indicates research be conducted to determine whether toxic phytochemicals exist in accessions of sunn hemp seed of interest and whether fiber can be extracted without contamination with toxic phytochemicals.

ACKNOWLEDGMENTS

The authors thank the S9 Regional Project and The University of Georgia for support of this Research, Jerry Davis, statistician, for his assistance with statistical analysis, and Beau Burton and Nina Carter for technical assistance.

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